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Letter to the Editor

Leptin levels in exhaled breath condensate from asthmatic children: a pilot study

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A cross-sectional analysis of the Third National Health and Nutrition Examination Survey (NHANES III) found that overweight children aged between 6 and 16 years had three times more asthma risk than their healthy weight peers (1). Another cross-sectional study supported the relationship between asthma and being overweight in childhood and showed that the prevalence of asthma was greater in overweight children (2).

Leptin, a 167-amino-acid peptide, is a recently discovered hormone which is believed to play a major role in the regulation of body weight. Recently, it has been reported that serum leptin levels are increased in asthmatic children (3). Leptin has also been reported to have a specific effect on T-lymphocyte responses. It has been hypothesized that leptin is involved in the regulation of asthmatic airway inflammation levels by regulating T-cell responses by polarizing Th cells toward a Th1 phenotype and by inhibiting immune responses, such as Th2 activation and may have a role in asthma (4). Mai et al. (5) found twice as high serum levels of leptin in overweight asthmatic children compared to overweight children without current asthma. Serum leptin levels are associated with asthma in adult women, independent of BMI (6). In children with asthma close associations have been shown between serum adiponectin and leptin and pulmonary function, but it has been suggested that they may play a regulatory role rather than being an etiologic mechanism of asthma development (7).

As very little is known about the role of leptin within the airways and lung tissue and asthma, non-invasive tools to investigate, such associations would allow epidemiological and longitudinal studies to be performed on a larger scale. Such studies are needed to confirm the relationships of leptin and asthma and improve our understanding of associations between asthma and being overweight.

Exhaled breath condensates (EBC) contain aerosolized airway lining fluid and volatile compounds from the airways and the lung periphery. The assessment of EBC compounds is recognized to be a potentially useful research tool in children for the non-invasive quantification of airway inflammation and metabolic processes in asthma and other airway diseases.

The aim of this pilot study was to evaluate whether leptin can be found in EBC and to investigate differences between condensate leptin levels in overweight and healthy weight children.

A total of 20 children (five overweight asthmatics, five healthy weight asthmatics, five overweight healthy and five healthy weight healthy controls), aged 6–15 years, were included in this study. We obtained data on weight and length (body mass index), lung function, fractional exhaled nitric oxide (FE_{NO}) and allergic sensitization by the means of skin prick testing using a panel of common allergens including house dust mite, mold mix, tree mix, grass mix, weed mix, cat and dog hair. Informed consent was taken from the parents.

Exhaled breath condensates were collected using the commercial EcoScreen breath condenser system (Jaeger, Wuerzburg, Germany). Approximately 1.5 mL of condensate was collected in 15 min and immediately stored at –70°C for later analysis. We standardized possible variables during and after EBC collection, according to the methodological recommendations of the ATS/ERS task force (8).

Condensate leptin levels were measured according to the instruction by the manufacturer using a commercially available ChemoKine™ Human leptin EIA kit (Chemicon International Inc., CA, USA) with a lower detection limit of 0.488 ng/mL. Leptin levels were measured in un-concentrated, native EBC and after concentrating the breath condensate 3-fold by freeze drying.

The patient characteristics, results of spirometry, $FE_{NO,}$ skin prick test and EBC leptin levels are summarized in Table 1.

Leptin levels were below the detection limit of the assay (0.488 ng/mL) in the native, un-concentrated EBC and in 3-fold concentrated samples and were therefore considered as non-detectable in any of the EBC samples.

We were not able to detect leptin in the EBC from asthmatic, healthy overweight and healthy weight children, even after 3-fold concentration of the EBC samples. We strictly followed the methodological recommendations of the ATS/ERS task force (8) and the instructions of the manufacturers and standardized possible variables during and after EBC

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Table 1 Patient characteristics and results of assessments.

	Asthmatics		Healthy	
	Healthy weight	Overweight	Healthy weight	Overweight
n (male/female)	4/1	3/2	4/1	3/2
Age	9.6±3.3	13.2±3.03	12.4±0.8	11.4±2.8
Sensitization[a]	5.8±4.0	3.4±3.3	NA	NA
BMI, kg/m^2	18.7±3.1	28.2±4.3	17.5±1.9	29.3±6.9
FEV_1, % pred	92.4±15.75	91.6±10.35	90.8±5.4	90.0±13.0
FE_{NO}, ppb	6.3±2.0	12.48±9.39	NA	NA
EBC leptin, ng/mL	<0.488	<0.488	<0.488	<0.488

All data in mean±standard deviation. Overweight: BMI-for-age ≥95th percentile. NA, not available. [a]Number of allergens. ppb, parts per billion.

collection. We therefore are confident that the non-detectable leptin levels in EBC were not due to a collection or measurement error. The assay we used is established for the measurement of leptin in biological fluids and is not restricted to serum.

There is still some controversy about the extent of proteins in EBC, but it is accepted that they are generally very low and often close to the detection limit of the assay used. More sensitive and/or specific assays, such as radio immunoassay or mass spectrometry may allow the measurement of leptin within EBC, but levels are expected to be extremely low.

A wide range of constituents in EBC have previously been measured and the analysis of EBC components has the potential to provide a non-invasive means of monitoring airway inflammation and cellular metabolism of the lung and airways. However, a range of important methodological issues need to be addressed for any EBC compound. These include repeatability, dilution, environmental contamination, effects of breathing patterns coating of the collector system and assay sensitivity (9, 10).

No leptin was detected in the EBC in native and 3-fold concentrated EBC. Exhaled breath condensates are not a useful tool to assess leptin metabolism in overweight and healthy weight asthmatic children.

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Conflict of interest statement

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References

1. Romieu I, Mannino DM, Redd SC, McGeehin MA. Dietary intake, physical activity, body mass index, and childhood asthma in the Third National Health and Nutrition Survey (NHANES III). Pediatr Pulmonol 2004;38:31–42.
2. Stingone JA, Ramirez OF, Svensson K, Claudio L. Prevalence, demographics, and health outcomes of comorbid asthma and overweight in urban children. J Asthma 2011;48:876–85.
3. Guler N, Kirerleri E, Ones U, Tamay Z, Salmayenli N, Darendeliler F. Leptin: does it have any role in childhood asthma? J Allergy Clin Immunol 2004;114:254–9.
4. Gurkan F, Atamer Y, Ece A, Kocyigit Y. Serum leptin levels in asthmatic children treated with an oral corticosteroid. Ann Allergy Asthma Immunol 2004;93:277–80.
5. Mai XM, Böttcher MF, Leijon I. Leptin and asthma in overweight children at 12 years of age. Pediatr Allergy Immunol 2004;15:523–30.
6. Sood A, Ford ES, Camargo CA Jr. Association between leptin and asthma in adults. Thorax 2006;61:300–5.
7. Kim KW, Shin YH, Lee KE, Kim ES, Sohn MH, Kim KE. Relationship between adipokines and manifestations of childhood asthma. Pediatr Allergy Immunol 2008;19:535–40.
8. Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, et al. ATS/ERS Task Force on Exhaled Breath Condensate. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J 2005;26:523–48.
9. Rosias PP, Dompeling E, Hendriks HJ, Heijnens JW, Donckerwolcke RA, Jöbsis Q. Exhaled breath condensate in children: pearls and pitfalls. Pediatr Allergy Immunol 2004;15:4–19.
10. Hunt J. Exhaled breath condensate: an evolving tool for noninvasive evaluation of lung disease. J Allergy Clin Immunol 2002;110:28–34.